

Diagnostic Accuracy of a Novel and Rapid Lipoarabinomannan Test for Diagnosing Tuberculosis Among People With Human Immunodeficiency Virus

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Background. The novel Fujifilm SILVAMP TB-LAM (FujiLAM) assay detects mycobacterial lipoarabinomannan in urine and has demonstrated superior sensitivity to the Alere Determine TB-LAM Ag (AlereLAM) assay for detection of tuberculosis among hospitalized people with human immunodeficiency virus (PWH). This is the first study to evaluate the assay among a broad population referred for antiretroviral therapy including both outpatients (mainly) and inpatients.

Methods. We assessed diagnostic accuracy of FujiLAM and AlereLAM assays in biobanked urine samples from a cohort of adults referred for antiretroviral therapy in Ghana against a microbiological and a composite (including clinical judgement) reference standard, and we assessed the association of FujiLAM test positivity with mortality.

Results. We evaluated urine samples from 532 PWH (462 outpatients, 70 inpatients). Against a microbiological reference standard, the sensitivity of FujiLAM was 74.2% (95% confidence interval [CI], 62.0–84.2) compared to 53.0% (95% CI, 40.3–65.4) for AlereLAM, a difference of 21.2% (CI, 13.1–32.5). Specificity was 89.3% (95% CI, 85.8–92.2) versus 95.6% (95% CI, 93.0–97.4) for FujiLAM and AlereLAM, a difference of –6.3% (95% CI –9.6 to –3.3). Specificity estimates for FujiLAM increased markedly to 98.8% (95% CI, 96.6–99.8) in patients with CD4 >100 cells/μL and when using a composite reference standard. FujiLAM test positivity was associated with increased cumulative risk of mortality at 6 months (hazard ratio, 4.80; 95% CI, 3.01–7.64).

Conclusions. FujiLAM offers significantly increased diagnostic sensitivity in comparison to AlereLAM. Specificity estimates for FujiLAM were lower than for AlereLAM but were affected by the limited ability of the reference standard to correctly diagnose tuberculosis in individuals with low CD4 counts.

Keywords. diagnostic accuracy; HIV; LAM; tuberculosis; urine.

Tuberculosis remains the leading cause of morbidity and mortality among people with human immunodeficiency virus (PWH) [1]. It is estimated that only 51% of tuberculosis cases among PWH are diagnosed and notified to health authorities [1]. This gap is partly explained by the limitations of available methods to diagnose tuberculosis in PWH, who are often

unable to produce sputum and have frequent extrapulmonary and/or paucibacillary disease [2, 3]. To improve tuberculosis case detection and treatment in PWH, a rapid test that does not rely on sputum is a key priority [1, 4].

In 2015, the World Health Organization (WHO) published recommendations for the urine lateral flow Alere Determine TB-LAM Ag test (AlereLAM; Abbott, Palatine, IL) to assist diagnosis of tuberculosis in PWH with a CD4 cell count ≤100 cells/μL or “who are seriously ill” [5]. The WHO policy was updated in 2019 to include recommendation for use of AlereLAM in a broader group of people, both in inpatient and outpatient settings [6]. The AlereLAM is simple to use and provides a result within 25 minutes. However, implementation of the test has been limited in parts due to its modest sensitivity [7]. A systematic review found a pooled sensitivity of 42% (95% credible interval [CrI], 31–55), increasing to 54% (CrI, 38–69) among people with CD4 ≤100 cells/μL, but reducing to 17% (CrI, 10–27) in people with CD4 >100 cells/μL [8]. Other factors that may have hindered

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implementation of AlereLAM in programmatic settings are the need of a reference scale card to interpret the AlereLAM test results and the emphasis on CD4 cell count to identify the target group for lipoarabinomannan (LAM) testing [9, 10].

The novel Fujifilm SILVAMP TB-LAM (FujiLAM; Fujifilm, Tokyo, Japan) also detects mycobacterial LAM in urine as a lateral flow test, through a 5-step process, with a result available within 1 hour [11, 12]. The FujiLAM test was developed through careful selection of a pair of high-affinity monoclonal antibodies for distinct *Mycobacterium tuberculosis* LAM epitopes present in urine samples of patients with tuberculosis [12]. The selection of detection antibodies in the FujiLAM test is further combined with a silver amplification step to increase visibility of the test lines [11]. FujiLAM has demonstrated superior sensitivity to AlereLAM in hospitalized patients with HIV (~70% vs 42%) [11].

This study is the first to assess diagnostic accuracy of FujiLAM for the detection of tuberculosis compared with AlereLAM among patient with HIV referred for antiretroviral therapy (ART), including mainly outpatients (expected to have lower pretest probability and higher CD4 cell count than inpatients), and to assess the predictive value of FujiLAM test positivity for mortality.

METHODS

Design, Setting, and Study Population

The diagnostic accuracy and predictive value of FujiLAM was evaluated in frozen urine samples stored from the DETECT HIV-TB study cohort of adults with human immunodeficiency virus (HIV) referred for ART to the Korle-Bu Teaching Hospital in Accra, Ghana [13, 14]. Participants for the DETECT HIV-TB study were recruited prospectively between January 2013 and March 2014 from the out- and inpatient departments at the Fevers unit. Adults were consecutively enrolled whether or not they reported tuberculosis symptoms if they met the following criteria: HIV-positive, ≥ 18 years, and referred for ART initiation (ie, WHO clinical stage 3 or 4, CD4 ≤ 350 cells/ μ L, or pregnant as per recommendations at the time of the study) [15]. Participants who were receiving treatment for tuberculosis or unable to produce any samples for mycobacterial testing (no sputum and no urine) were excluded.

Demographic, clinical, and routine laboratory data including CD4 cell count were collected, the research team systematically collected urine and spontaneously expectorated sputum samples upon enrollment, and participants were asked to bring in an additional early morning sputum sample. At 6-month follow up, medical records were reviewed for vital status, loss to follow up, ART, and tuberculosis treatment status. If medical records were unavailable, contact with the participants or next of kin was attempted by phone.

Informed consent was obtained from all participants, and the study was approved by the Institutional Review Board of University of Ghana Medical School and the Danish National Committee

on Health Research Ethics. We followed the Standards for the Reporting of Diagnostic accuracy studies (STARD) criteria [16].

Procedures

Urine specimens collected as part of the DETECT HIV-TB cohort were stored at -20°C at the Department of Medical Microbiology, University of Ghana and shipped on dry ice to the Research Institute of Tuberculosis/Japan Anti-Tuberculosis Association in Tokyo, Japan for LAM testing between January and March 2019. At the time of testing, frozen urine aliquots were thawed to ambient temperature and mixed manually. FujiLAM and AlereLAM testing were done from the same aliquot.

FujiLAM testing was done in accordance with the manufacturer's instructions that involve a 5-step procedure as previously described [11] (see [Supplementary Figure S1](#)). In

Table 1. Tuberculosis Diagnostic Classification

Category	Description
Definite TB	Any culture or any Xpert (baseline) positive for MTB ≥ 1 Positive culture (solid, liquid, sputum) and confirmed MTB complex at baseline OR ≥ 1 Positive Xpert (sputum or urine) at baseline
Possible TB	Any patient not meeting definite TB or not TB classification who is started on TB treatment or has positive laboratory findings on follow up Empiric TB treatment started by the healthcare provider OR Positive sputum culture and/or sputum Xpert and/or sputum smear on follow up
Not TB	All microscopy, culture, and Xpert tests negative for MTB, not started on TB treatment, recovers, and has negative follow-up tests All cultures negative (sputum, blood, including follow-up where available) AND All Xpert negative (sputum, urine, including follow-up where available) AND All smear microscopy negative (sputum including follow-up where available) AND Treatment not initiated by healthcare providers AND Improvement or full recovery of symptoms at 2 months of follow up in the absence of TB treatment
Unclassifiable	All participants that do not fall into groups "definite TB," "not TB" or "possible TB" ie, No symptom resolution at follow up (same or worse) in patients with a mycobacterial work-up that are negative at baseline or in follow up at 2 months OR Loss to follow up at 2 months (patients with a mycobacterial work-up that is negative at baseline) OR Passed away (patients with a mycobacterial work-up that is negative at baseline) OR Baseline smear microscopy positive but culture and Xpert negative

Abbreviations: MTB, *Mycobacterium tuberculosis* complex; TB, tuberculosis.

brief, approximately 200 µL urine was added to the reagent tube up to the indicator line, mixed, and incubated for 40 minutes at ambient temperature. After mixing again, 2 drops of sample

were added to the test strip. Then, 2 buttons were pressed sequentially within 3–10 minutes for the silver amplification. The final result was available within 50–60 minutes.

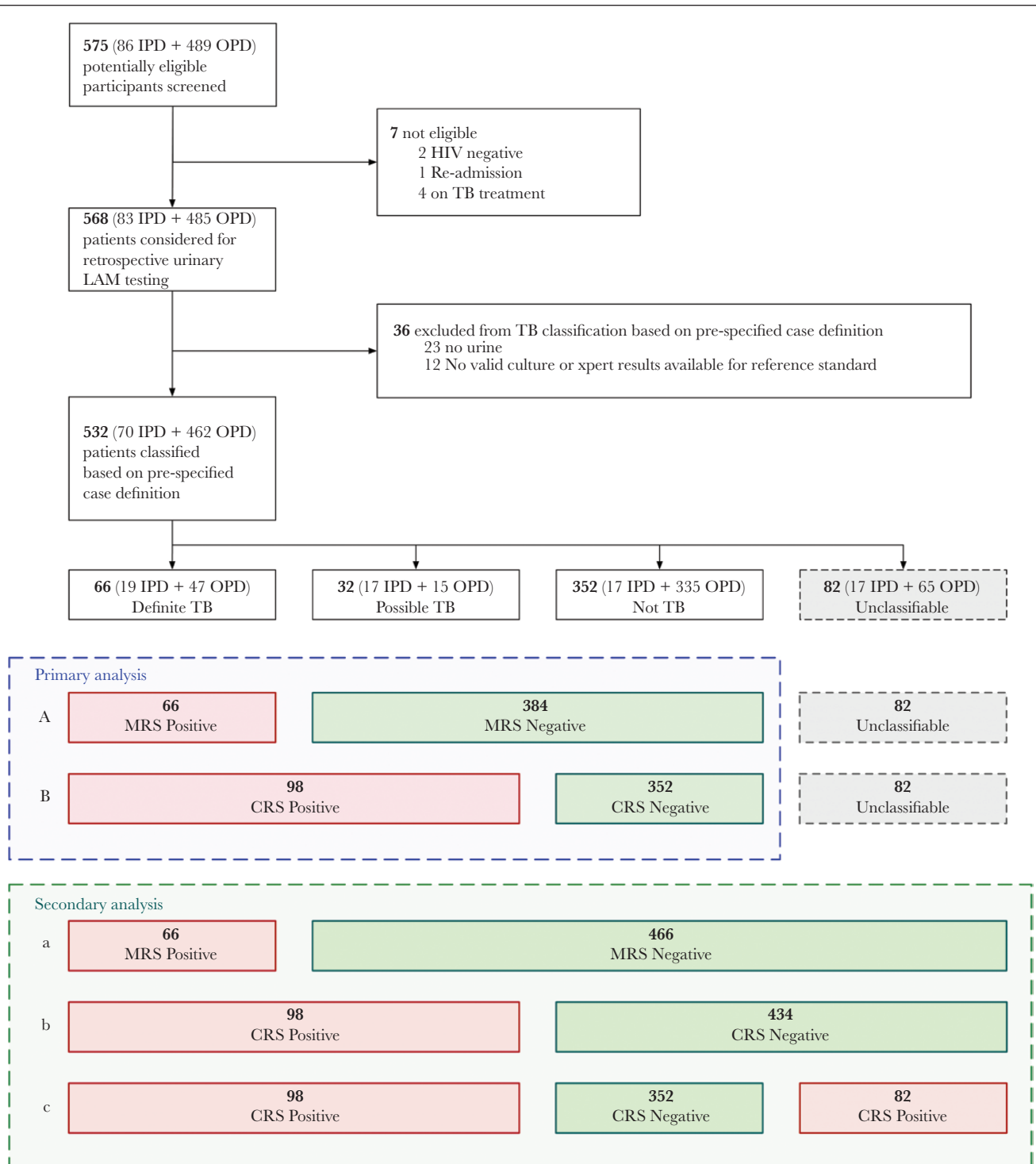


Figure 1. Flow diagram of study population and classification. (Analysis A) Primary diagnostic accuracy analysis including all human immunodeficiency virus (HIV)⁺ against a Microbiological Reference Standard (MRS). (Analysis B) Primary diagnostic accuracy analysis including all HIV + against a Composite Reference Standard (CRS). (Analysis a) Secondary sensitivity analysis including all HIV⁺ with unclassifiable reclassified as MRS negative. (Analysis b) Secondary sensitivity analysis including all HIV + with unclassifiable reclassified as CRS negative. (Analysis c) Secondary sensitivity analysis including all HIV⁺ with unclassifiable reclassified as CRS positive. IPD, inpatients; OPD, outpatients; TB, tuberculosis.

AlereLAM testing was done according to the test's package insert [17]. In brief, 60 µL urine was applied to the sample pad on the test strip and the result was read after 25 minutes. We used the updated reference scale card with 4 color band intensities to grade the test as negative or grade 1, 2, 3, or 4 and considered the recommended grade 1 cutoff point as the positivity threshold [17].

The AlereLAM and FujiLAM test results were read independently by 2 readers blinded to their counterpart's observations, to the test result of the respective other test, to patient status as well as the results of the tuberculosis reference tests. In the event of discordance, the 2 readers reinspected the test to establish a final consensus result. In case of test failure, the test was repeated once.

For reference standard testing, sputum samples were processed as previously described [13, 14]. Testing included smear microscopy for acid-fast bacilli using both Ziehl-Neelsen and Auramine O staining, sputum culture for mycobacteria using both solid Löwenstein-Jensen medium and the BACTEC mycobacteria growth indicator tube (MGIT) liquid culture system (BD Diagnostics, Sparks, MD), and Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA). Positive mycobacterial isolates were sent to the German National Reference Centre for Mycobacteria in Borstel for speciation using the GenoType Mycobacterium CM/AS (Hain Lifescience, Nehren, Germany) assay and analysis of 16S ribosomal ribonucleic acid gene. In addition, urinary Xpert testing was performed using 6 mL biobanked urine

thawed and centrifuged. After removal of the supernatant, the pellet was resuspended in 1 mL sterile phosphate-buffered saline and mixed with the Xpert sample reagent.

Diagnostic Classification and Statistical Analysis

We categorized participants according to the tuberculosis diagnostic categories shown in Table 1 while blinded to the LAM test results. Definite tuberculosis included participants that had a culture (sputum) or Xpert (sputum or urine) positive result for *M tuberculosis* complex in any of the samples obtained at baseline; not tuberculosis included participants that were alive at 2 months follow up with negative microscopy, culture, and Xpert tests at baseline and follow up and no empirical tuberculosis treatment; for "possible" tuberculosis, the participant did not meet the criteria for "definite" tuberculosis but was started and responded to tuberculosis treatment based on clinical grounds or had a positive test result at follow up; "unclassifiable" was determined if the participant did not meet any of the above categories.

Figure 1 outlines diagnostic classification of participants and analysis. In primary analysis, using a microbiological reference standard (MRS), participants categorized as definite tuberculosis were considered reference standard positive, and participants with not tuberculosis and possible tuberculosis were considered as negative. In a composite reference standard (CRS), participants with possible tuberculosis were reclassified as positive together with definite tuberculosis. Participants that

Table 2. Patient Characteristics

Key Characteristics of Study Population					
	All n = 532 (%)	Definite TB n = 66 (%)	Possible TB n = 32 (%)	Not TB n = 352 (%)	Unclassifiable ^a n = 82 (%)
Enrollment Site					
Outpatients	462 (86.8)	47 (71.2)	15 (46.9)	335 (95.2)	65 (79.3)
Inpatients	70 (13.2)	19 (28.8)	17 (53.1)	17 (4.8)	17 (20.7)
Median age in years (IQR)	38 (31–45)	37 (29–43)	37 (34–44)	39 (31–45)	38 (32–45)
Female	354 (66.5)	39 (59.4)	19 (59.4)	245 (69.6)	51 (62.2)
Median CD4 cell count per µL (IQR)^b					
CD4 ≤100 cells/µL	152 (44–349)	108 (43–190)	45 (12–179)	216 (76–410)	56 (16–220)
CD4 >100 cells/µL	192 (37.7)	32 (50.0)	19 (63.3)	97 (28.4)	44 (59.5)
CD4 >100 cells/µL	318 (62.3)	32 (50.0)	11 (36.7)	245 (71.6)	30 (40.5)
Median BMI (IQR)	21 (18–23)	18 (16–20)	18 (16–22)	21 (19–25)	20 (17–22)
Positive WHO symptom screen ^c	457 (86.2)	63 (95.5)	30 (93.8)	295 (83.8)	69 (86.3)
Previous history of TB ^d	35 (6.6)	5 (7.6)	3 (9.4)	22 (6.3)	5 (6.3)
FujiLAM positive	97 (18.2)	49 (74.2)	14 (43.8)	27 (7.8)	7 (8.5)
AlereLAM positive	57 (10.7)	35 (53.3)	8 (25.0)	9 (2.6)	5 (6.1)
Vital Status at 6 Months					
Alive	372 (69.9)	32 (48.5)	15 (46.9)	321 (91.2)	4 (4.9)
Died	71 (13.4)	22 (33.3)	13 (40.6)	7 (2.0)	29 (35.4)
LTFU	89 (16.7)	12 (18.2)	4 (12.5)	24 (6.8)	49 (59.8)

Abbreviations: BMI, body mass index; IQR, interquartile range; LTFU, loss to follow up; TB, tuberculosis; WHO, World Health Organization.

^aUnclassifiables were excluded from primary analysis.

^bCD4 count missing for 22 participants.

^cWHO symptoms score missing for 2 participants.

^dTB history missing for 4 participants.

were unclassifiable were excluded from the primary analysis. The effect on performance of including the unclassifiable participants was assessed in a secondary analysis.

Descriptive analyses were used to characterize the study population. We determined sensitivity and specificity with 95% confidence interval (CI) as well as positive predictive value (PPV) and negative predictive value and likelihood ratios of AlereLAM and FujiLAM against both a microbiological and CRS for the study population overall and for predefined subgroups. The 95% CI around differences between FujiLAM and AlereLAM sensitivities and specificities were calculated according to the Tango [18] method, and the difference was considered significant if the 95% CIs did not include zero. Interreader agreement for each of the FujiLAM test and AlereLAM test results, respectively, was determined by Cohen's kappa coefficient. To evaluate the association between LAM positivity (by FujiLAM and AlereLAM) and mortality at 2 and 6 months, we used Cox regression analysis and reported the unadjusted hazard ratio (HR) with 95% CI. We constructed Kaplan-Meier curves to examine risk of mortality after 6 months in the population overall and among definite tuberculosis cases, and we compared data stratified by FujiLAM and AlereLAM test results with the log-rank test. All analyses were conducted using STATA version 15.1 software.

RESULTS

Study Population and Tuberculosis Diagnostic Status

Of 575 individuals screened for participation in the DETECT HIV-TB study cohort, 568 were considered eligible for retrospective LAM testing, and 532 had a urine sample and a reference standard result available to enter the study (Figure 1). Of 532 eligible participants, 82 participants (15.4%) were unclassifiable according to tuberculosis diagnostic status and excluded from our primary analysis. The main reason for participants to be unclassifiable was lost to follow up as listed in Supplementary Table S1. Of the 450 participants included in the primary analysis, 66 (14.7%) were classified as definite tuberculosis, 32 (7.1%) were classified as possible tuberculosis, and 352 (78.2%) were classified as not tuberculosis.

Participants had a median CD4 cell count of 152 (interquartile range [IQR], 44–349), and the majority (462 [86.8%]) were outpatients (Table 2). A total of 1937 sputum and urine samples were collected from participants (mean, 3.6 samples/participant). This provided basis for a total of 2448 reference standard test results (mycobacterial solid/liquid culture and Xpert; mean, 4.6 tests/participant) including 2018 tests on sputum samples and 430 tests on urine samples. Lowenstein-Jensen (LJ) contamination rate was 6.0% (9 of 501) for spot sputum samples and 7.0% (31 of 442) for early morning samples. For MGIT, contamination rates were 12.4% (62 of 501) for spot sputum and 17.1% (76 of 442) for early morning samples. For 3 participants, sputum was not available or contaminated in both spot

Table 3. Sensitivity and specificity of FujiLAM versus AlereLAM against MRS (A) and CRS (B)

A	MRS	Test	N	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	TB%	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
	All	FujiLAM	450	49	41	17	343	74.2% (62.0–84.2)	89.3% (85.8–92.2)	14.7%	54.4% (43.6–65.0)	95.3% (92.5–97.2)	7.0 (5.0–9.6)	0.3 (0.2–0.4)
		AlereLAM	450	35	17	31	367	53.0% (40.3–65.4)	95.6% (93.0–97.4)	14.7%	67.3% (52.9–79.7)	92.2% (89.1–94.6)	12.0 (7.1–20.1)	0.5 (0.4–0.6)
		ΔSn and ΔSn						21.2% (13.1–32.5)	–6.3% (–9.6 to –3.3)					
B	CRS	Test	N	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	TB%	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)
	All	FujiLAM	450	63	27	35	325	64.3% (54.0–73.7)	92.3% (89.0–94.9)	21.8%	70.0% (59.4–79.2)	90.3% (86.7–93.1)	8.4 (5.7–12.4)	0.4 (0.3–0.5)
		AlereLAM	450	43	9	55	343	43.9% (33.9–54.3)	97.4% (95.2–98.8)	21.8%	82.7% (69.7–91.8)	86.2% (82.4–89.4)	17.2 (8.7–34.0)	0.6 (0.5–0.7)
		ΔSn and ΔSn						20.4% (11.8–30.0)	–5.1% (–8.4 to –2.3)					

Abbreviations: AlereLAM, Alere Determine TB LAM Ag assay; CI, confidence interval; CRS, composite reference standard; FN, false negative; FP, false positive; FujiLAM, Fujifilm SILVAMP TB LAM assay; HIV, human immunodeficiency virus; LAM, liparabinomannan; LR+, likelihood ratio (+); LR-, likelihood ratio (-); MRS, microbiological reference standard; NPV, negative predictive value; PPV, positive predictive value; Sp, specificity; TB%, tuberculosis prevalence; TN, true negatives; TP, true positives.

* (A) Diagnostic accuracy using a MRS: Definite tuberculosis were considered reference standard positive. Not tuberculosis and Possible tuberculosis were considered reference standard negative. (B) Diagnostic accuracy using a CRS: Definite tuberculosis and Possible tuberculosis were considered reference standard positive. Not tuberculosis were considered reference standard negative.

and early morning samples for both LJ and MGIT, and tuberculosis diagnostic categorization relied on the urine Xpert result.

Comparison of Accuracy of Fujifilm SILVAMP TB-LAM and Alere Determine TB-LAM Ag

Against an MRS, FujiLAM detected 49 and AlereLAM 35 of 66 of participants with definite tuberculosis, giving an overall sensitivity of 74.2% (49 of 66; 95% CI, 62.0–84.2) for FujiLAM versus 53.0% (35 of 66; 95% CI, 40.3–65.4) for AlereLAM (difference of 21.2%; CI, 13.1–32.5) (Table 3). Among outpatients, FujiLAM sensitivity was 68.1% (32 of 47; 95% CI, 52.9–80.9) compared with AlereLAM sensitivity of 44.7% (21 of 47; 95% CI, 30.2–59.9); difference of 23.4% (95% CI, 13.6–37.2) (Table 4).

The sensitivity of both tests increased with lower CD4 cell counts. The sensitivity in participants with CD4 \leq 100 cells/ μ L was 84.4% (27 of 32; 95% CI, 67.2–94.7) for FujiLAM compared with 65.6% (21 of 32; 95% CI, 46.8–81.4) for Alere LAM (difference of 18.8%; 95% CI, 6.0–35.3) (Table 5). The difference in sensitivity was greatest for participants with CD4 $>$ 200 cells/ μ L in favor of FujiLAM (26.6%; 95% CI, 0.8–52.0), but overall numbers were small (Table 4). Against a CRS, overall sensitivity decreased to 64.3% (63 of 98; 95% CI, 54.0–73.7) for FujiLAM and to 43.9% (43 of 98; 95% CI, 33.9–54.3) for AlereLAM (retaining a difference of 20.4%; 95% CI, 11.8–30.0) (Table 3).

Against an MRS, the specificity was 89.3% (343 of 384; 95% CI, 85.8–92.2) for FujiLAM versus 95.6% (367 of 384; 95% CI, 93.0–97.4) for AlereLAM (difference of –6.3%; 95% CI, –9.6 to –3.3) (Table 3). FujiLAM specificity was lowest in participants with CD4 \leq 100 cells/ μ L (69.8%; 81 of 116; 95% CI, 60.6–78.0) but increased markedly to 98.8% (253 of 256; 95% CI,

96.6–99.8) in participants with CD4 $>$ 100 cells/ μ L, where it was comparable to AlereLAM specificity 99.2% (254 of 256; 95% CI, 97.2–99.9; difference of –0.4% [95% CI, –2.5 to 1.5]) (Table 5).

Specificity increased when using a CRS to 92.3% (325 of 352; 95% CI, 89.0–94.9) for FujiLAM and to 97.4% (343 of 352; 95% CI, 95.2–98.8) for AlereLAM (Table 3). Of note, 25 of 27 FujiLAM-positive participants classified as tuberculosis negative against the CRS had a CD4 cell count below 100, and 22% (6 of 27) died or were lost to follow up after 2 months (Supplementary Table S2).

Against an MRS restricted to the results of urine Xpert testing (n = 419), we found that FujiLAM was positive in 83.3% (15 of 18) of participants with a positive urine Xpert result (sensitivity), whereas AlereLAM was positive in 72.2% (13 of 18) of participants. Additional analysis of FujiLAM and AlereLAM accuracy by smear status is shown in Supplementary Table S3. Our secondary analysis, reclassifying the unclassifiable, is reported in the Supplementary Table S4.

For FujiLAM testing, 8 of 532 (1.5%) required a second test to produce valid results. For AlereLAM, 2 of 532 (0.4%) required a second test for a valid result. The following 2 types of errors were reported for FujiLAM: (1) insufficient volume of reducing reagent released after pressing button 1 so that the liquid did not reach the end of the test strip, and (2) negative control line. Interreader agreement between the readers was high for both tests (FujiLAM 98.5% [kappa 0.9487; Standard Error (SE) 0.043], AlereLAM 99.3% [kappa 0.961; SE 0.043]) (Supplementary Table S5).

Nontuberculous Mycobacteria and Fujifilm SILVAMP TB-LAM Positivity

Nontuberculous mycobacteria (NTM) were cultured from sputum of 53 of 532 participants (10.0%; 95% CI, 7.7–12.9)

Table 4. Sensitivity and specificity of FujiLAM versus AlereLAM against MRS (A) and CRS (B) by patient status

MRS	Test	N	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Outpatients	FujiLAM	397	32	33	15	317	68.1% (52.9–80.9)	90.6% (87.0–93.4)
	AlereLAM	397	21	11	26	339	44.7% (30.2–59.9)	96.9% (94.4–98.4)
	Δ Sn and Δ Sp						23.4% (13.6 to 37.2)	–6.3% (–9.7 to –3.5)
Inpatients	FujiLAM	53	17	8	2	26	89.5% (66.9–98.7)	76.5% (58.8–89.3)
	AlereLAM	53	14	6	5	28	73.7% (48.8–90.9)	82.4% (65.5–93.2)
	Δ Sn and Δ Sp						15.8% (–3.7 to 37.6)	–5.9% (–23.7 to 11.8)
CRS	Test	N	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Outpatients	FujiLAM	397	39	26	23	309	62.9% (49.7–75.8)	92.2% (88.8–94.9)
	AlereLAM	397	24	8	38	327	38.7% (26.6–51.9)	97.6% (95.3–99.0)
	Δ Sn and Δ Sp						24.2% (15.3 to 36.2)	–5.4% (–8.7 to –2.6)
Inpatients	FujiLAM	53	24	1	12	16	66.7% (49.0–81.4)	94.1% (71.3–99.9)
	AlereLAM	53	19	1	17	16	52.8% (35.5–69.6)	94.1% (71.3–99.9%)
	Δ Sn and Δ Sp						13.9% (–2.9 to 31.0)	0.0% (–22.7 to 22.7)

Abbreviations: AlereLAM, Alere Determine TB LAM Ag assay; CI, confidence interval; CRS, composite reference standard; FN, false negatives; FP, false positives; FujiLAM, Fujifilm SILVAMP TB LAM assay; LAM, lipoarabinomannan; MRS, microbiological reference standard; Sn, sensitivity; Sp, specificity; TN, true negatives; TP, true positives.

(A) Diagnostic accuracy using a MRS: Definite tuberculosis were considered reference standard positive. Not tuberculosis and Possible tuberculosis were considered reference standard negative. (B) Diagnostic accuracy using a CRS: Definite tuberculosis and Possible tuberculosis were considered reference standard positive. Not tuberculosis were considered reference standard negative.

Table 5. Sensitivity and specificity of FujiLAM versus AlereLAM against MRS (A) and CRS (B) by CD4 strata

	Test	N	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
0–100 cells/ μ L	FujiLAM	148	27	35	5	81	84.4% (67.2–94.7)	69.8% (60.6–78.0)
	AlereLAM	148	21	14	11	102	65.6% (46.8–81.4)	87.9% (80.6–93.2)
	Δ Sn and Δ S _n						18.8% (6.0 to 35.3)	–18.1% (–27.6 to –9.0)
>100 cells/ μ L	FujiLAM	288	20	3	12	253	62.5% (43.7–78.9)	98.8% (96.6–99.8)
	AlereLAM	288	12	2	20	254	37.5% (21.1–56.3)	99.2% (97.2–99.9)
	Δ Sn and Δ S _n						25.0% (11.6 to 42.1)	–0.4% (–2.5 to 1.5)
0–200 cells/ μ L	FujiLAM	237	39	35	10	153	79.6% (65.7–89.8)	81.4% (75.1–86.7)
	AlereLAM	237	29	15	20	173	59.2% (44.2–73.0)	92.0% (87.2–95.5)
	Δ Sn and Δ S _n						20.4% (11.5 to 33.6)	–10.6% (–17.0 to –4.8)
>200 cells/ μ L	FujiLAM	199	8	3	7	181	53.3% (26.6–78.7)	98.4% (95.3–99.7)
	AlereLAM	199	4	1	11	183	26.7% (7.8–55.1)	99.5% (97.0–100.0)
	Δ Sn and Δ S _n						26.6% (0.8 to 52.0)	–1.1% (–3.9 to 1.0)
101–200 cells/ μ L	FujiLAM	89	12	0	5	72	70.6% (44.0–89.7)	100.0% (95.0–100.0)
	AlereLAM	89	8	1	9	71	47.1% (23.0–72.2)	98.6% (92.5–100.0)
	Δ Sn and Δ S _n						23.5% (–0.8 to 47.3)	1.4% (–3.7 to 7.5)
CRS	Test	N	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
0–100 cells/ μ L	FujiLAM	148	38	24	13	73	74.5% (60.4–85.7)	75.3% (65.5–83.5)
	AlereLAM	148	28	7	23	90	54.9% (40.3–68.9)	92.8% (85.7–97.0)
	Δ Sn and Δ S _n						19.6% (6.0 to 33.9)	–17.5% (–27.6 to –8.1)
>100 cells/ μ L	FujiLAM	288	21	2	22	243	48.8% (33.3–64.5)	99.2% (97.1–99.9)
	AlereLAM	288	12	2	31	243	27.9% (15.3–43.7)	99.2% (97.1–99.9)
	Δ Sn and Δ S _n						20.9% (11.0 to 35.2)	0.0% (–1.9 to 1.9)
0–200 cells/ μ L	FujiLAM	237	50	24	22	141	69.4% (57.5–79.8)	85.5% (79.1–90.5)
	AlereLAM	237	36	8	36	157	50.0% (38.0–62.0)	95.2% (90.7–97.9)
	Δ Sn and Δ S _n						19.4% (9.0 to 31.0)	–9.7% (–16.1 to –3.9)
>200 cells/ μ L	FujiLAM	199	9	2	13	175	40.9% (20.7–63.6)	98.9% (96.0–99.9)
	AlereLAM	199	4	1	18	176	18.2% (5.2–40.3)	99.4% (96.9–100.0)
	Δ Sn and Δ S _n						22.7% (0.4 to 43.4)	–0.6% (–3.1 to 1.6)
101–200 cells/ μ L	FujiLAM	89	12	0	9	68	57.1% (34.0–78.2)	100.0% (94.7–100.0)
	AlereLAM	89	8	1	13	67	38.1% (18.1–61.6)	98.5% (92.1–100.0)
	Δ Sn and Δ S _n						19.0% (0.6 to 40.0)	1.5% (–4.0 to 7.9)

Abbreviations: AlereLAM, Alere Determine TB LAM Ag assay; CI, confidence interval; CRS, composite reference standard; FN, false negatives; FP, false positives; FujiLAM, Fujifilm SILVAMP TB LAM assay; LAM, lipoarabinomannan; MRS, microbiological reference standard; Sn, sensitivity; Sp, specificity; TN, true negatives; TP, true positive.

(A) Diagnostic accuracy using a MRS: Definite tuberculosis were considered reference standard positive. Not tuberculosis and Possible tuberculosis were considered reference standard negative. (B) Diagnostic accuracy using a CRS: Definite tuberculosis and Possible tuberculosis were considered reference standard positive. Not tuberculosis were considered reference standard negative.

(Supplementary Table S6). Of these, NTM were cultured in 2 sputum specimens for 9 of 53 (17.0%) cases and in a single sputum sample for the remaining cases. Among not tuberculosis participants with NTM isolated, FujiLAM was negative in 25 of 27 (92.6%) cases and positive in 2 of 27 (7.4%) with the slow-growing *Mycobacterium intracellulare* and *Mycobacterium avium* cultured from sputum. Both of these participants had low CD4 cell count (2 and 65) and survived 6 months of follow up without any treatment for mycobacteria, suggesting against a disseminated systemic infection. Among possible tuberculosis participants with NTM isolated, FujiLAM was positive in 6 of 7 (85.7%) cases, and clinical characteristics suggested a severely sick population with extreme immunosuppression (median CD4 of 3 cells/ μ L; IQR, 2–5) and high mortality (50%; 3 of 6 died within 6 months). Data for AlereLAM are presented in Supplementary Table S6.

Association of Lipoarabinomannan Test Positivity and Mortality

By 6 months, 71 (13%) of all participants had died and an additional 89 (17%) were lost to follow up. The median time to death for FujiLAM-positive participants was 30 days (IQR, 8–91) compared with 13 days (IQR, 7–39) for AlereLAM. FujiLAM was positive in 49.3% (35 of 71) of participants who had died by 6 months versus 35.2% (25 of 71) for AlereLAM (difference 14%; 95% CI, 2.1–26.0). Mortality proportions for all participants and those with definite tuberculosis stratified by FujiLAM and AlereLAM status are presented in Supplementary Table S7. A positive FujiLAM test result was associated with higher risk of death at 2 and 6 months compared with FujiLAM negative (Table 6). AlereLAM-positive participants had a higher HR for death than FujiLAM. The Kaplan-Meier mortality curves are shown in Figure 2.

Table 6. Cox Regression Analyses for Mortality at 2 and 6 Months According to FujiLAM and AlereLAM Status Among All Participants (n = 532) and Among Definite TB Participants (n = 66)

Population	Unadjusted Hazard Ratio (95% CI)	P Value
At 2 Months, All Participants (n = 532)		
FujiLAM negative	Ref.	
FujiLAM positive	3.6 (2.0–6.2)	<.001
AlereLAM negative	Ref.	
AlereLAM positive	6.7 (3.8–11.7)	<.001
At 2 Months, Definite TB (n = 66)		
FujiLAM negative	Ref.	
FujiLAM positive	6.2 (0.8–46.4)	.077
AlereLAM negative	Ref.	
AlereLAM positive	8.7 (2.0–38.0)	.004
At 6 Months, All participants (n = 532)		
FujiLAM negative	Ref.	
FujiLAM positive	4.8 (3.0–7.6)	<.001
AlereLAM negative	Ref.	
AlereLAM positive	6.2 (3.8–10.1)	<.001
At 6 Months, Definite TB (n = 66)		
FujiLAM negative	Ref.	
FujiLAM positive	3.7 (0.9–16.0)	.075
AlereLAM negative	Ref.	
AlereLAM positive	5.2 (1.7–15.3)	.003

Abbreviations: CI, confidence interval; Ref., reference; TB, tuberculosis.

DISCUSSION

In this first tuberculosis diagnostic accuracy study of the urinary FujiLAM point-of-care test among adult outpatients with HIV, we demonstrate superior sensitivity compared with that of AlereLAM in our study population overall and across CD4 strata (sensitivity gain ranging from 15% to 25% difference). FujiLAM alone could detect 74% of definite tuberculosis cases. Specificity was lower for FujiLAM than for AlereLAM, but the majority of FujiLAM false-positive results were observed among participants with CD4 ≤ 100 cells/ μ L, whereas specificity of the 2 tests was comparable in participants with CD4 > 100 cells/ μ L. Specificity increased using a CRS.

Results from our study are in line with the findings of a recent assessment of FujiLAM among hospitalized patients with HIV and mirror the same trend of highest FujiLAM sensitivity in participants with low CD4 cell count [11]. We further demonstrate a significant sensitivity gain of using FujiLAM compared with AlereLAM among outpatients and participants with higher CD4 cell count. Overall, sensitivity was above the minimum WHO suggested target of 65% for rapid nonsputum TB tests and approaching the optimal requirement of 80% [4]. The estimated specificity was low in participants with advanced immunosuppression (CD4 ≤ 100 cells/ μ L), but otherwise it met the optimal target of 98% against an MRS [4].

Our MRS was based on cultures from 2 sputum samples and Xpert testing of sputum and urine. Although culture is considered the best available reference standard for tuberculosis, it is not a perfect reference standard due to the paucibacillary

nature and the common extrapulmonary and disseminated disease among PWH, particularly among patients with low CD4 count [3, 19, 20]. Moreover, culture, and in particular liquid culture (MGIT), is prone to contamination, as also found in our study. Our reference standard was further limited by sputum Xpert not being available for all participants, no use of sputum induction, and use of a low volume of urine (6 mL) for urinary Xpert testing. This may have impaired the quality and diagnostic yield of the reference standard and would be expected to disproportionately affect a more sensitive test [19, 21]. By recognizing these limitations to an otherwise comprehensive reference standard, we may have misclassified a truly positive FujiLAM result as a false positive. Misclassification may in part explain the low FujiLAM specificity among participants with CD4 ≤ 100 cells/ μ L, where the tuberculosis diagnosis is hardest to ascertain. Low specificity among patients with low CD4 cell count was also noted for AlereLAM test in this study as well as in systematic reviews of AlereLAM diagnostic accuracy studies [8, 22]. These findings highlight the challenge of assessing diagnostic specificity for a test that is not site-specific, and that it is important to recognize that specificity estimates may suffer from reference-standard bias.

We could not determine whether heterogeneity in specificity estimates was fully attributable to misclassification bias. In studies of AlereLAM, we previously found some signs of cross-reactivity between AlereLAM and NTM [13, 23], and others also suggested that disseminated NTM disease may cause false-positive AlereLAM results [24, 25]. In analytical studies of the antibodies used in FujiLAM, there was no observed cross-reactivity to rapid-growing NTM [12]. In our study, isolation of NTM in sputum was common and likely represented a mix of NTM contaminants (eg, the vast majority had a single sputum with NTM isolation) and some with true NTM pulmonary/disseminated disease. The majority of not tuberculosis with NTM cultured were FujiLAM negative (25 of 27), whereas 2 participants were FujiLAM positive both with slow-growing NTMs isolated. Given the limitations of our reference standard, it is possible that the participants may have had mixed infection with *M tuberculosis* that was missed. Participants with possible tuberculosis and sputum NTM isolation (mainly slow-growing) had advanced immunosuppression, high mortality, and negative urine Xpert to suggest possible pulmonary/disseminated NTM disease, and the majority (6 of 7) were FujiLAM positive. Although this raises concerns that clinically important slow-growing NTMs among PWH may affect FujiLAM test result and contribute to low specificity estimate, it needs to be evaluated in studies with a higher quality of reference standard to discriminate between isolated tuberculosis disease, pulmonary/disseminated NTM diseases, and the possibility of a mixed infections with tuberculosis and NTM. The background prevalence of NTM disease relative to the burden of tuberculosis among PWH must also be considered when assessing the PPV

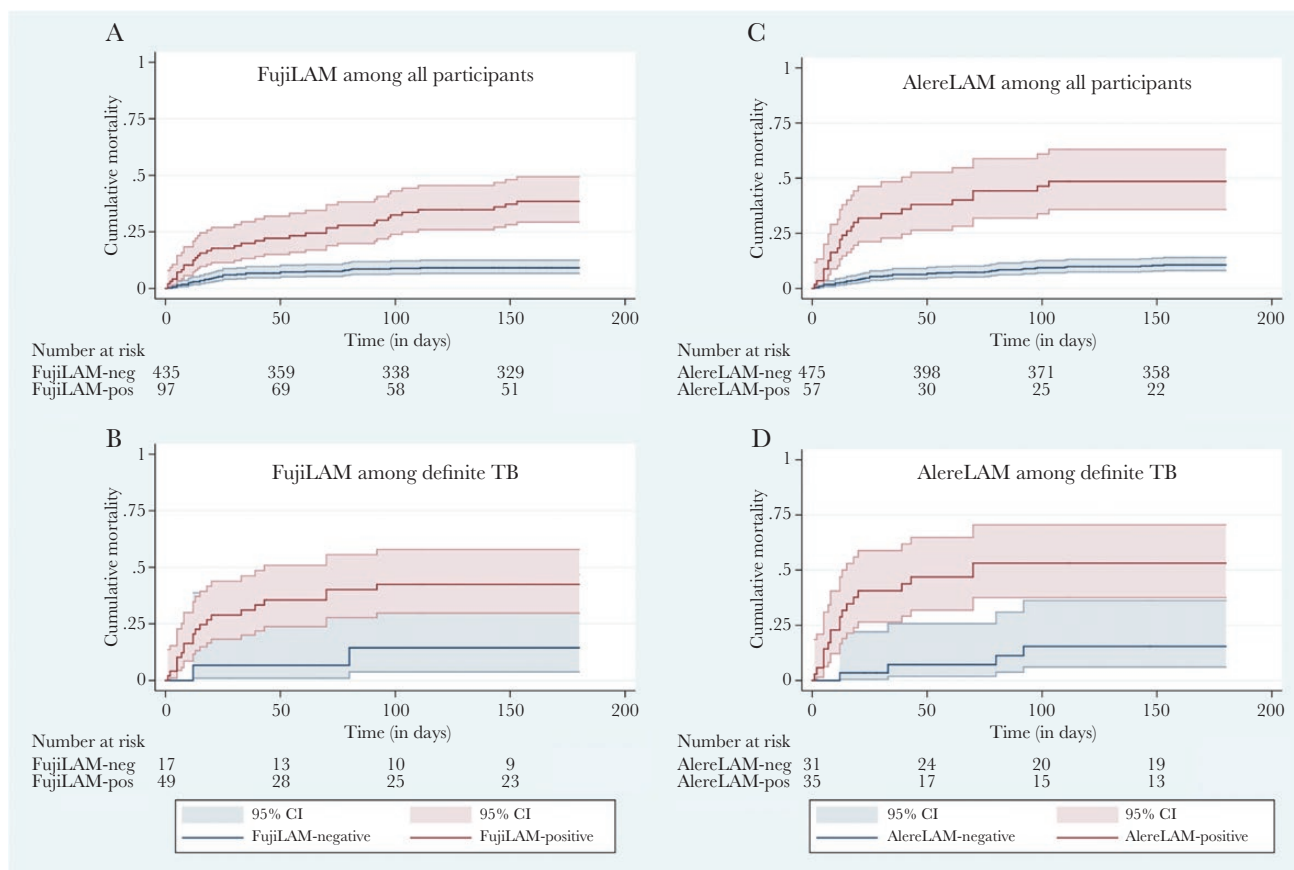


Figure 2. Kaplan-Meier curves for cumulative 6-month mortality stratified by FujiLAM and AlereLAM, respectively. Kaplan-Meier curves shown for (A) FujiLAM among all participants ($n = 532$; log rank < 0.001); (B) FujiLAM among definite tuberculosis (TB) participants ($n = 66$; log rank = 0.054); (C) AlereLAM among all participants ($n = 532$; log rank < 0.001); (D) AlereLAM among definite TB participants ($n = 66$; log rank = 0.001).

of FujiLAM results (and implications of possible cross-reactivity) [26]. Concerns about specificity may need to be assessed with the potential biases in mind and also considering the potential benefits of increased sensitivity for a population in which there may be no other means of making a diagnosis of tuberculosis and mortality of tuberculosis is high.

The turnaround time is longer for the FujiLAM test (50–60 minutes) than the AlereLAM (25 minutes) test and requires additional steps in the workflow. On the other hand, the FujiLAM test interpretation is easier because no reference scale card, such as that used for the AlereLAM test, is required [9]. Implications of these differences on the feasibility and acceptance of the FujiLAM test in clinical practice and for integration in existing algorithms should be addressed in future studies.

A positive FujiLAM test predicted mortality in our study population and identified a higher proportion of participants who died than the AlereLAM test. We do not know whether FujiLAM testing could have prevented some of these deaths, as demonstrated for AlereLAM in clinical trials [27, 28]. We speculate that because the FujiLAM test is developed to detect LAM at a lower concentration than AlereLAM [12], FujiLAM detects LAM from early stages of tuberculosis disease where patients

are less sick and survive the follow-up period. Previous quantitative LAM studies have demonstrated that LAM concentration positively correlates with poor prognosis, which supports this explanation [29, 30].

The strengths of this study are a large and representative cohort of well characterized PWH with follow up of 6 months. We have already discussed the limitations of our reference standard. Other limitations include that only ART-naive patients were included in the study and the retrospective testing of stored samples; however, a recent study showed high agreement of FujiLAM test results using fresh versus biobanked samples [31]. We could not classify 82 (15%) participants according to our predefined diagnostic classification mainly due to loss to follow up, but sensitivity analysis including the unclassifiable did not affect specificity, and the higher sensitivity of FujiLAM compared with AlereLAM was maintained. Prospective studies in clinical setting is the next step needed to evaluate the diagnostic accuracy of FujiLAM. Future studies should consider systematic sampling of multiple specimens from pulmonary and extrapulmonary sites to improve the quality of the reference standard and assess the effect of clinically important disseminated/pulmonary NTM disease.

CONCLUSIONS

In conclusion, with significantly higher sensitivity compared with AlereLAM, the FujiLAM test has the potential to improve early detection and treatment of tuberculosis among PWH and translate this into reduced morbidity and mortality. Prospective studies of FujiLAM in clinical settings with a comprehensive mycobacterial reference test are now needed.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Potential conflicts of interests. R. S., A. M., S. G. S., and E. M. are employed by FIND, and T. B. and C. M. D. were employed at FIND during the conduct of the study. T. B. further reports patents in the field of lipoarabinomannan detection. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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